essential. For example, non-complementary nucleotide residues can be attached to the 5' end of the primer, with the remainder of the primer sequence being complementary to the template. Alternatively, non-complementary nucleotide residues or a stretch of non-complementary nucleotide residues can be interspersed into a primer, provided that the primer sequence has sufficient complementarity with the sequence of the template to hybridise therewith and thereby form a template for synthesis of the extension product of the primer.

[0075] "Isolation" of a nucleic acid is to be understood to mean a nucleic acid which has generally been separated from other components with which it is naturally associated or linked in its native state. Preferably, the isolated polynucleotide is at least 50% free, more preferably at least 75% free, and more preferably at least 90% free from other components with which it is naturally associated. The degree of isolation expressed may relate to purity from interfering substances.

[0076] "Isolation" of a biosample refers to "forward isolation", wherein the biosample container may be handled without exposure to infectious agent, and to "reverse isolation", wherein the sample is not contaminated during handling. "Biosafe" thus has a second dimension, assurance of the quality of the sample.

[0077] Any method of nucleic acid amplification may be suitable for use in embodiments of the present invention. For example, an isothermal amplification technique may be particularly applicable in the amplification of nucleic acids in the present invention. One such isothermal technique is LAMP (loop-mediated isothermal amplification of DNA) and is described in Notomi, T. et al. Nucl Acid Res 2000 28:e63.

[0078] Strand Displacement Amplification (SDA) is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, i.e., nick translation (Walker et al. Nucleic Acids Research, 1992:1691-1696). A similar method, called Repair Chain Reaction (RCR), involves annealing several probes throughout a region targeted for amplification, followed by a repair reaction in which only two of the four bases are present. The other two bases can be added as biotinylated derivatives for easy detection. A similar approach is used in SDA. Target specific sequences can also be detected using a cyclic probe reaction (CPR). In CPR, a probe having 3' and 5' sequences of non-specific DNA and a middle sequence of specific RNA is hybridised to DNA that is present in a sample. Upon hybridisation, the reaction is treated with RNase H, and the products of the probe identified as distinctive products that are released after digestion. The original template is annealed to another cycling probe and the reaction is repeated.

[0079] An exemplary nucleic acid amplification technique is the polymerase chain reaction (referred to as PCR) which is described in detail in U.S. Pat. Nos. 4,683,195, 4,683,202 and 4,800,159, Ausubel et al. Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), and in Innis et al., ("PCR Protocols", Academic Press, Inc., San Diego Calif., 1990). Polymerase chain reaction methodologies are well known in the art. Briefly, in PCR, two primer sequences are prepared that are complementary to regions on opposite complementary strands of a target sequence. An excess of deoxynucleoside triphosphates are added to a reaction mixture along with a DNA polymerase, e.g., Taq polymerase. If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the marker sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the template to form reaction products, excess primers will bind to the template and to the reaction products and the process is repeated. By adding fluorescent intercalating agents, PCR products can be detected in real time.

[0080] Another nucleic acid amplification technique is reverse transcription polymerase chain reaction (RT-PCR). First, complementary DNA (cDNA) is made from an RNA template, using a reverse transcriptase enzyme, and then PCR is performed on the resultant cDNA.

[0081] Another method for amplification is the ligase chain reaction ("LCR"), disclosed in EPO No. 320 308. In LCR, two complementary probe pairs are prepared, and in the presence of the target sequence, each pair will bind to opposite complementary strands of the target such that they abut. In the presence of a ligase, the two probe pairs will link to form a single unit. By temperature cycling, as in PCR, bound ligated units dissociate from the target and then serve as "target sequences" for ligation of excess probe pairs. U.S. Pat. No. 4,883,750 describes a method similar to LCR for binding probe pairs to a target sequence.

[0082] Qβ Replicase, may also be used as still another amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence that can then be detected.

[0083] Still further amplification methods, described in GB Application No. 2 202 328, and in PCT Application No. PCT/US89/01025, may be used in accordance with the present invention. In the former application, "modified" primers are used in a PCR-like, template- and enzyme-dependent synthesis. The primers may be modified by labelling with a capture moiety (e.g., biotin) and/or a detector moiety (e.g., enzyme). In the latter application, an excess of labelled probes are added to a sample. In the presence of the target sequence, the probe binds and is cleaved catalytically. After cleavage, the target sequence is released intact to be bound by excess probe. Cleavage of the labelled probe signals the presence of the target sequence.

[0084] Other nucleic acid amplification procedures include transcription-based amplification systems (TAS), including nucleic acid sequence based amplification (NASBA) and 3SR (Kwoh et al., 1989, Proc. Natl. Acad. Sci. U.S.A., 86: 1173; Gingeras et al., PCT Application WO 88/10315). In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a clinical sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer which has target specific sequences. Following polymerisation, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat denatured again. In either case the single stranded DNA is made fully double stranded by addition of second target specific primer, followed by polymerisation. The double-stranded DNA molecules are then multiply transcribed by an RNA polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse transcribed into single stranded DNA, which is then converted to double stranded DNA, and then transcribed once again with an RNA poly-